Intracorporeal Autologous Hepatocyte Matrix Implant for the Treatment of Chronic Liver Disease: A Modified Clinical Phase I Study

by Siufui Hendrawan

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Intracorporeal Autologous Hepatocyte Matrix Implant for the Treatment of Chronic Liver Disease: A Modified Clinical Phase I Study

Hans U. Baer[1], Siufui Hendrawan[2], Suryadi The[3], Syafruddin AR. Lelosutan[4], Salim G[2], Toni Lindl[5], Stephanie Mathes[6], Ursula Graf-Hausner[7], Ursula Weber[1], Randall Watson[8] and Barlian Sutedja[3]*

[1]Center of Abdominal Surgery, Hirslanden Clinic, University of Bern, Switzerland

[2]Tarumanagara Human Cell Technology Laboratory, Tarumanagara University, School of Medicine, Indonesia

[3]Department of Surgery, Gading Pluit Hospital, Indonesia

[4]Department of Internal Medicine, Gading Pluit Hospital, Indonesia

[5]IAZ - Institute for Applied Cell Culture, Germany

[6]University of Zurich, Center of Dental Medicine, Switzerland

[7]Zurich University of Applied Sciences, Tissue Engineering/Drug Development (TEDD), Switzerland

[8]Medical Writer, Limmatstrasse, Switzerland

Abstract

Background and Aim: Cultured human autologous hepatocyte matrix implants have been used to treat chronic liver cirrhosis with some success and are a promising method to counter liver damage. The options afforded by hepatocyte transplant are of special importance as this may prolong or improve the lives of patients waiting for a transplant. We assessed the safety of implanted scaffold cultured hepatocytes upon patient survival in a modified phase 1 trial.

Methods: We present here the first ethically approved in human study of autologous hepatocyte matrix implantation for the treatment of predominantly viral hepatitis induced cirrhosis. The study was performed in patients with longstanding liver disease at a single center in Jakarta, Indonesia. Liver segments and pancreatic tissue from each patient were taken and processed to single hepatocytes and islets of Langerhans, respectively. The single cells were co-seeded onto PLA scaffolds, cultured, and transplanted back into the patient. We performed pre-implantation diagnosis and measured clinical disease severity scores (CTP and MELD) and liver function (albumin, bilirubin, ammonia, cholinesterase levels). In our small cohort, the procedure was generally safe.

Results: The small patient number prevented any statistical assessment of efficacy. For some patients we observed clinically relevant improvements of liver function. Some patients showed improvements in stamina, endurance to physical stress, reduced frequency of hospitalization and incidence of encephalopathy, GI bleeding, ascites, and esophageal varices.

Conclusion: Although limited in power for statistical significance, the present work presents sufficient data to warrant pursuing a phase 2 follow-up study.

Keywords: Chronic liver disease; Autologous hepatocyte matrix implant; PLLA matrix

Abbreviations

CTP: Child-Turcotte-Pugh; GI: Gastrointestinal; ICU: Intensive-Care Unit; MELD: Model for End-stage Liver Disease; PGA: Poly-Glycolic Acid; PLA: Poly-Lactic Acid

Introduction

Chronic liver cell injury resulting in liver cirrhosis has become a major health burden in many societies. In western countries, alcohol abuse has been identified as a primary cause of such cirrhosis. In Asian countries, viral hepatitis is a more prevalent factor leading to cirrhosis and eventual hepatic failure. Patients suffering hepatic failure and cirrhosis-induced metabolic disorders are known to present very complex cases [1]. Often, patients suffer from impaired coagulation, altered

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Correspondence:
Hans U. Baer, Center of Abdominal Surgery, Klinik Hirslanden, Witellikerstrasse 40, 8052 Zurich, University of Bern, Inselspital Bern, Switzerland, Tel: 41443873070; Fax: 41443873090;
E-mail: hans.baer@bluewin.ch

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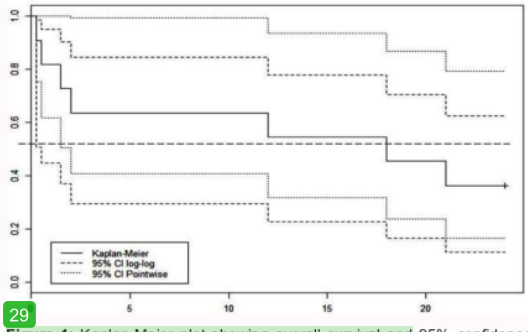

Figure 1: Kaplan-Meier plot showing overall survival and 95% confidence intervals calculated using two different methods. The wide confidence intervals prevent the determination of statistically significant conclusions on survival.

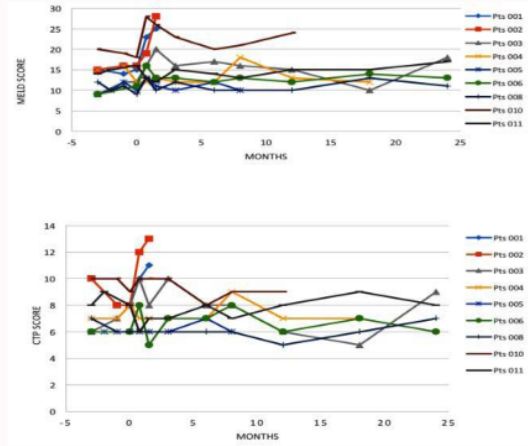

Figure 2: MELD and CTP scores for all patients surviving beyond 1 month post-surgery.

consciousness and cerebral function, a heightened risk of multiple organ failure, and even sepsis.

In the western world liver transplantation remains the only curative option in most cases [1]. Unfortunately, even in modern and well-funded healthcare systems a severe shortage of donors means that many patients die on waiting lists [2]. In many developing nations, the sophisticated transplant programs that are common in developed nations are in a fledgling state or do not exist. Consequently the prognosis in terms of morbidity and mortality is poor depending upon the regional situation [3]. A method to prolong life or improve patient health would increase the likelihood of receiving a transplant and could have resounding implications upon overall survival in this patient group.

Hepatocyte transplantation is emerging as a promising method to repair liver damage [4]. Early attempts used cells from heterologous cell donors injected into the splenic or portal vein [5,6]. However, poor cell engraftment and viability have been identified as limiting factors in 'traditional' hepatocyte transplantation [1,7].

Attempts to improve engraftment and to implant cells that are more durable and viable have resulted in advancements in tissue

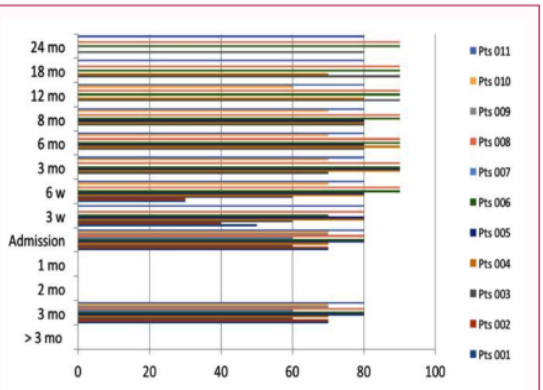

Figure 3: Karnofsky scale through time.

engineering. In more recent years, such efforts have increased the potential for implantation success of exogenous tissue matrices seeded with autologous hepatocytes [8].

A number of these exogenous tissue matrices utilize Poly-Lactic Acid (PLA) and Poly-Glycolic Acids (PLG) to form a temporary 3 dimensional scaffold to support cell growth and enable the generation of in vitro tissue. PLA and PLG have a long history of clinical use as components of sutures and implants. They are clinically tested and safe, with minimal toxicity [9].

Typically, the three dimensional scaffolds are seeded with cells and cultured to create an engineered tissue which can then be implanted. Uyama et al. found that the viability of the hepatocyte tissue implants can be greatly enhanced by co-seeding the hepatocytes with pancreatic cells, more specifically islets of Langerhans, which showed the role of hepatotropic stimulation in hepatocyte transplantation [10].

Animal studies

Uyama et al. used 3D PLA scaffolds to culture rat hepatocyte cells [11]. This work showed superior cell density and function when compared to 2D cultures. Moreover, engineered hepatocyte scaffolds, when implanted into rats, were fully viable for a period of at least six months [10].

It has been determined that for meaningful tissue function to be achieved, the implants need to become properly vascularized, and undertake metabolic exchange. The mesentery of the small bowel has been found to provide a suitable implant location to establish such vascularization and integration [12]. However, the number of animal studies is limited and the generalizability of their findings unknown. Therefore such studies are not necessarily transferable to human applications [13].

Human implantation

Cultured human autologous hepatocyte matrix implants have been used in non-viral cirrhotic patients [14]. In the only study known to the authors, excluding the present work, 57 patients who developed liver cirrhosis through alcoholism were treated with autologous hepatocyte implants. For the majority of these patients, liver-related blood values remained stable or improved 12 months after implantation.

Undoubtedly, the results of this work look promising; however, the study was limited to alcohol-related liver damage, little is known

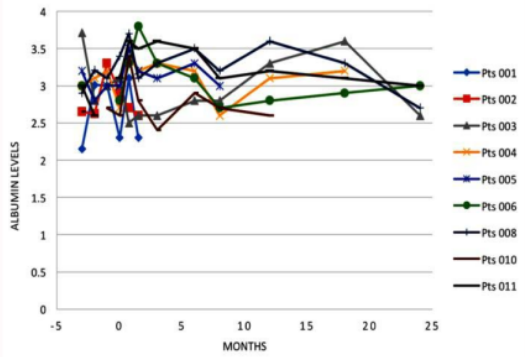

Figure 4: Albumin levels for each patient through time.

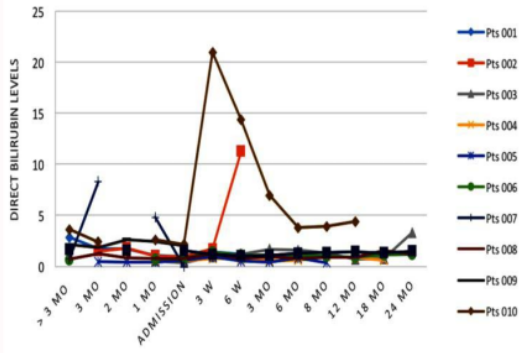

Figure 6: Direct bilirubin levels through time.

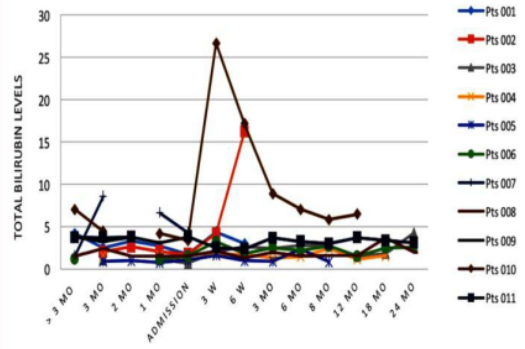

Figure 5: Total bilirubin levels for each patient through time.

about the exact protocol, and it is unclear if the study was carried out in a manner consistent with best practice.

Rationale

In the developing world currently, it is unlikely that cirrhotic patients will receive a liver transplant. Being able to offer these patients a "bridge to transplant", that is, a method to prolong or improve health and thereby increase the eventual likelihood of a transplant would be especially welcome.

Here we present the first ethically approved in-human study of autologous hepatocyte matrix implantation for the treatment of predominantly viral hepatitis induced cirrhosis. This modified Phase I study sets out to investigate safety, with the intention to extend to a Phase II trial in the future.

Methods

Patients

Patients with longstanding liver disease under the care of two specialists from a single center in Jakarta, Indonesia, were selected using the inclusion criteria.

Informed consent & ethical approval

To ensure full patient understanding and allow patient family consultation and considered decision making, patients were required to provide informed consent on two separate occasions at least 4 weeks apart. Briefly, information brochures were given to potential patients after the first consultation meeting. These materials included the written description of the study, an integrated informed consent agreement and the official declaration of approval from the Ethics Committee.

Surgical technique and scaffold generation

The surgical procedure consisted of two separate procedures in theatre under general anesthesia; firstly, the collection of liver and pancreatic tissue, to provide hepatocytes and Islets of Langerhan's cells, and secondly, the implantation of the cultured implant matrices into the small bowel mesentery.

Tissue harvest

Under strict sterile technique, a 4 cm × 4 cm × 4 cm liver segment was removed from liver segment III. From the pancreas, a biopsy of 3 mm × 3 mm × 3 mm was taken. Collected tissues were treated according to the custom organ transplant protocol with cold Custodiol® transplant solution and triple-bag sterile packing with crushed ice, and transported to the laboratory in a monitored cold box (0°C to 4°C). A transport time of less than two hours with constant temperature is accepted as safe for tissue survival.

Engineered tissue scaffolds

The generation of the tissue scaffolds [15] will be the subject of a secondary publication. Briefly, under strict Biosafety Level 2 conditions at the Tarumanagara Human Cell Technology Laboratory, harvested tissues were treated to liberate hepatocytes and islets of Langerhans as described in the literature [14]. Cells, after quantification, were c seeded onto 20 mm × 4 mm PLA disk scaffolds (Phrontier SARL, 2 rue Saint Clair, 76490 Caudebec en Caux, France) pre coated with collagen type 1 to allow cell adherence. For each patient, 20 scaffolds were seeded, each with 1-2 million cells in 300 µL William E medium completed with 10% autologous patient serum. Seeded scaffolds were cultured in 12-well cell cul plates with 0.7 mL William E with 10% autologous serum and incubated at 37°C with 5% CO_2 and 95% relative humidity for no less than 60 hr before implantation into the patient.

Re-implantation

Approximately three days after the initial laparotomy, the subcostal incision was reopened and the small bowel mesentery moved into the wound. A 2 cm incision in the serosa was made and a cavity with a well vascularized bed was formed to provide space for

two implants. The serosa was closed with interrupted 5/0 sutures. Fifteen to twenty implants were used per patient. The abdominal wall was closed as soon as the matrices had been successfully inserted.

Assessment

Safety, the primary outcome, was directly assessed through patient survival.

The following outcomes and pre-operative variables were recorded for 24 months after the procedure or until death if earlier. For some patients, detailed historical data were also available. The following parameters were also recorded.

- Pre-implantation diagnosis
- Clinical disease severity scores
- CTP score
- MELD scores
- Albumin, bilirubin, ammonia and cholinesterase

Statistical analysis

The small number of patients precludes rigorous statistical analyses relating to survival [11]. However, the primary objective of this phase I trial was to determine safety and not efficacy.

Results

Fifty patients were screened in the Department of Surgery, Gading Pluit Hospital, Indonesia, for possible inclusion in the study. Eleven patients were included in the final cohort. Baseline assessment data for each patient is given in Table 1.

Primary outcome-safety and survival

All patients survived the first operation (cell harvesting). Two patients died following the second operation: one died one week after surgery from severe Gastrointestinal (GI) bleeding caused by esophageal varices, and the other 2 weeks post-surgery from hepatorenal syndrome.

During the 24-month follow up, a further 5 patients died; none were deemed procedure related. At 24 months, four patients remained alive with good clinical indicators. Mortality and cause of death are listed in Table 2. After 24 months, one of the surviving patients returned liver function test results consistent with deteriorating liver function caused by hepatitis C. Overall survival is shown in the Kaplan-Meier plot in Figure 1 and 95% CIs were calculated using both the point wise and the preferred log-log method.

Clinical and laboratory markers of liver function

Clinical assessments of patients were made throughout the follow up period and detailed notes maintained. CTP and MELD scores for each patient are shown in Figure 2. Individual CTP scores at 6, 12 and 24 months post-surgery were relatively stable compared to baseline. Individual MELD scores at 6, 12 and 24 months were elevated in a majority of patients.

The Karnofsky subjective scale showed a trend to slight improvement in some of the patients (Figure 3).

Albumin levels

Albumin levels are shown in Figure 4. Of the seven patients alive at 6 months, six showed improved albumin levels compared with time of admission. At 12 months, all living patients had improved albumin levels [6], and at 24 months, one patient had improved albumin levels, one was stable, and two had deteriorated.

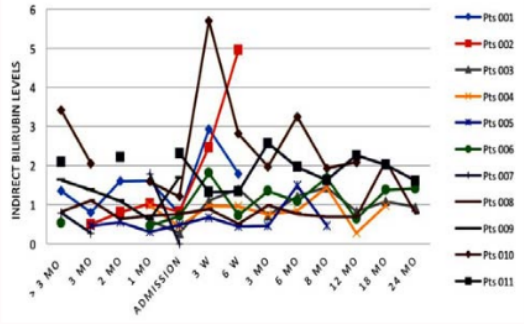

Figure 7: Indirect bilirubin levels through time.

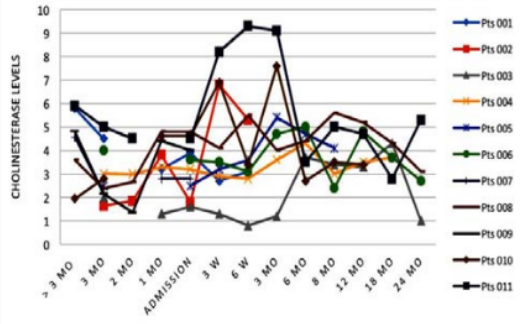

Figure 8: Cholinesterases levels through time.

Bilirubin-total, direct and indirect (conjugated)

At six months, 5 of 7 patients had elevated total bilirubin levels (Figure 5). Although there was some stabilization at 12 months, at 24 months, bilirubin levels were still above baseline in 3 of 4 patients. Similarly, indirect and direct bilirubin levels fluctuated but remained elevated in the majority of patients at all stages, with 3 of 4 patients having elevated levels by 24 months (Figures 6 and 7).

Cholinesterase

The fluctuation through time of cholinesterase levels is shown in Figure 8 for each patient. In general, patients surviving at 18 months showed general improvement in cholinesterase.

Ammonia

Levels fluctuated in the follow-up period, but were not significantly elevated or lowered.

Other results

At 24 months, all patients still on study stated they had greater stamina and better endurance to physical stress, although this was not measured objectively. Subjective patient-reported observation indicated a greater sense of well being, even amongst those who eventually died during the study. Incidence of encephalopathy and GI bleeding were also diminished among the remaining patients. And in two patients there was improvement in ascites or esophageal varices.

Discussion

The liver is the central organ in metabolism. Impairment of

Table 1: Patient baseline assessment including histological results of the 11 patients, using the Metavir Scoring System.

	Gender	Age at Study entry	Diagnosis	Disease duration (y)	Viral Load (iu/mL)	Cirrhosis Grade				CTP Score Point	MELD Score
						Metavir	Lobular	piecemeal	Fibrosis		
Median (range)	64% M	58 (43-67)		Since Diag.6 (2-20) Cirr. 4(1-7)	6.4×10^{3} 9.5 t0 3×10^{5}	3(2-3)	1(0-2)	3(2-3)	4(4)	8(6-9)	15(9-21)
Mean		56.8		6.6	7.1×10^{4}					7.7	14
Patient				Hep (Cirrhosis)							
001	M	58	Hep. C	4(4)	Undetected	3	2	3	4	8	15
002	M	52	Hep. B	2.5(1)	9.5×10^{0}	2	1	2	4	8	16
003	M	56	Hep. B	20(1)	8.4×10^{2}	3	2	3	4	8	12
004	M	57	Hep. C	10(7)	Undetected	2	1	2	4	8	12
005	F	67	Hep. C	4(4)	3.5×10^{3}	2	1	2	4	6	10
006	M	43	Hep. C	6(6)	9.2×103	2	1	2	4	6	11
007	F	58	Non B Non C	7	N.A	3	1	3	4	9	21
008	F	59	Hep. C	2(2)	3×10^{5}	3	1	3	4	6	9
009	F	60	Hep. C	8(5)	1.4×10^{5}	3	0	3	4	9	15
010	M	59	Hep. C	7(7)	1.6×10^{5}	3	0	3	4	9	18
011	M	56	Hep. B	2(1)	1×10^{5}	2	1	2	4	8	16

Table 2: Clinical outcome, mortality, and cause of death for enrolled patients.

Patient identifier	Cause of death	Time after surgery (months)	Procedure related?
001	Death from liver failure exacerbation	2	Liver failure exacerbation
002	Death from upper GI bleeding	1.5	Gastrointestinal bleeding from esophageal varices
003		24	Improved ascites and stamina but deteriorated again after 18 months post implantation
004	Death from heart attack (?)	21	Deteriorating portal hypertension with refractory ascites, pleural effusions and abdminal hernia. Sudden cardiac arrest
005	Death from cholangitis sepsis	11	1 year post-implantation. Cholangiosepsis due to common bile duct stones
006		24	Improved stamina, still active working
007	Death from hepatorenal syndrome	0.5	2 weeks post-implantation surgery (procedure related)
008		24	Improved stamina, no varices
009	Death from upper GI bleeding	0.25	Esophageal varices, severe GI bleeding (deemed procedure related)
010		18	1.5 years post implantation acute liver failure
011	Death from liver failure	24	No recurrence bleeding & stable stamina in 2 years post implantation

liver function can have severe effects that, among other symptoms, may manifest as jaundice, esophageal varices and bleeding, portal hypertension, ascites, primary liver failure, cachexia and encephalopathy. The variety of clinical symptoms is linked to the central role of hepatocytes, which perform a multitude of tasks such as albumin and cholinesterase synthesis, conversion and storage of glucose, detoxification of drugs, and excretion of bile and bile soluble substances like bilirubin. In the course of research to find new techniques that might alleviate the severe shortage of donor livers, we tested here evaluation-blinded, non-randomized autologous hepatocyte matrix implantation as a method to redress the balance in treating liver disease without the need for liver transplantation.

Safety

Primary outcome

The primary outcome of this phase I trial was to assess the safety of the autologous cultured hepatocyte matrix transplantation technique in patients suffering from severe liver cirrhosis due primarily to hepatitis. To fulfill this criterion, the implants must be well tolerated and should not induce an inflammatory reaction or malignant transformation of the implanted cells. Additionally, the overall risk for patients should be analogous to, or lower than, that of orthotopic liver transplantation, which is the alternative treatment. The inclusion of an orthotropic liver transplant control group in this setting was unfeasible due to the extremely limited availability of such treatment in the study clinic. Further, the inclusion of placebo control is ethically unsupportable in this setting.

Despite strict inclusion and exclusion criteria, seven patients died due to hepatorenal syndrome, GI bleeding, and or liver failure. Of these, five were disease related and two were considered to be procedure-related by ethical committee review. Of the two procedure-related deaths, one was due to acute hepatorenal syndrome deemed to have been triggered by hepatic insult induced by the surgery. Surgical interventions in patients with higher MELD scores (≥ 15) are very risky with estimates of 90-day mortality exceeding 25.4% for all surgery types and even higher for procedures involving hepatic resection [16]. The patient in question suffered from non-viral hepatitis (the only case in this trial) and had a pre-operative MELD score of 21, the highest in the cohort. All patients who died within 90 days of the procedure had a MELD score of 15 or greater.

The other procedure-related death occurred through fundus

varices bleeding 1 week after the surgical procedure. The classification of this patient death as procedure-related has been questioned, and it might have been preventable under slightly different follow-up protocol requiring closer GI monitoring.

Indeed, GI bleeding is of significant concern for many cirrhotic patients as a result of portal hypertension. In our cohort, 8 of 11 patients had a history of upper GI bleeding, and one other patient on the trial died from upper GI bleeding on day post-implant. This death was classified as disease related and not as a result of the procedure. The results of the first seven patients were reviewed by both the ethical committee and advisory board. It was decided to continue with the study but to revise the exclusion criteria to exclude patients with a MELD score above 15.

Overall, mortality was not directly correlated with the procedure but the impact and burden of the procedure did influence the outcome of some patients. We found no mortality for those patients in CTP A but for CTP B, mortality was closely correlated with the MELD score. Mortality was mostly seen in those with MELD score of 15 or above. Finally, it is important to note the general level of illness amongst the cohort. Of 50 patients screened for inclusion in the study, 13 were initially selected, however two of these were subsequently excluded after a secondary assessment and one of these patients died only one day after being discharged following the second assessment.

Impact of viral load on mortality

Ten of the 11 patients in the study cohort suffered from viral hepatitis: 3 type B and 7 type C. The inclusion criteria excluded patients with cirrhosis and active chronic viral hepatitis. In normal daily practice, many physicians set a viral load limit up to 10^3 to 10^4 iU/mL. Our cohort included patients with up to 3×10^5 iU/mL, but we also included three hepatitis patients with undetectable or extremely low viral counts. We saw no correlation between 30-day mortality and viral load for either hepatitis B or hepatitis C. However, we did observe indicators of constant inflammation through slightly elevated SGOT/SGPT/Gamma GT levels, and we suspect viral load to be a cause of this inflammation and to ultimately play a role in continued liver fibrosis. In the case of hepatitis B, we were able to rely on oral antivirus medication; however we were not so fortunate for hepatitis C cases.

Post-mortem

Religious protocol prevented any post-mortem examinations. Postmortem examinations may have revealed interesting data on potential malignancy issues and on the ultimate long-term viability of the implanted tissue.

Clinical Benefit

Overall survival

The performance of the procedure in terms of clinical benefit is difficult to assess. This is primarily a result of the limited numbers enrolled in the study. Overall survival, as distinct from procedure-related mortality, and shown in the Kaplan-Meier plot, suggests a median survival of around 18 months, however the broad confidence intervals associated with such a small cohort negate objective value of any such figure.

Clinical indicators

Clinical indicators of general health and prognosis were stable and did not, in general, show marked improvement. The CTP and MELD scores were relatively stable; however a slight increase in MELD scores was noticed across the cohort. It should be noted that the baseline MELD scores for patients were relatively high with a median of 15 (9-21) and as such, most patients would be expected to exhibit a rise in MELD score over time due to natural disease progression. We therefore believe the slow rise in MELD score is attributable to the natural disease course and do not note any correlation of individual patient's MELD scores with their health state. It must be remembered that the MELD score is not a measure of vitality, rather a measure of the need for transplantation.

During the initial 3-month pre-operative period the CTP scores decreased for 5 of 11 patients, and increased for two patients. This decrease probably reflects overall improvement in liver function in these patients and is likely due to the cyclic nature of the cirrhosis. Patients were treated during the study with best practice clinical support, standard medical therapy for hepatitis, and nutritive control. Patients were monitored during the pre-treatment phase to exclude those who were spontaneously recovering. The CTP score is a clinical score however and not a prognostic score, and as such all patients enrolled had high MELD scores and were in relatively poor condition. Despite the slight improvement in the CTP score for some patients, the improvement was not of sufficient magnitude to warrant exclusion from the study.

Laboratory values

Bilirubin levels are an indicator for bile excretion and therefore liver function. All patients showed a temporary increase in bilirubin immediately following surgery, a common occurrence following any surgical intervention in cirrhotic patients. Aside from 2 patients who showed sudden increases in total bilirubin after surgery, one subsequently died at 6 weeks and the other recovered. Bilirubin levels remained mostly stable throughout the course of the study with the highest increase being a doubling of the baseline level. When examined in further detail, direct bilirubin, reflecting the true excretion function of bile from the liver, remained at or around baseline levels, except in the two aforementioned patients. One further patient showed a late increase in direct bilirubin from 18-24 months coupled with a general deterioration of health. Indirect bilirubin levels fluctuated slightly more than direct bilirubin. Except for the two cases of significant elevation, bilirubin fluctuations were not clinically relevant.

Serum albumin levels gradually improved across all patients who survived beyond 6 months; however a general trend for stable or slightly decreasing serum albumin developed after 12-18 months amongst surviving patients. One patient, with a 20 year history of hepatitis B requiring repeated albumin infusions prior to treatment, showed continuously improving albumin levels after treatment up to the end of the study where a sharp decrease in albumin and concomitant increase in bilirubin and rising hepatitis C viral levels were recorded. Overall, the increase in albumin is clinically significant in the first year; however, the limited cohort does not allow statistically significant conclusions to be made.

Laboratory results for ammonia, as a reflection of liver metabolism and excretory function, fluctuated throughout the course of the study but remained within allowable levels and appeared to show no correlation with patient daily performance. Cholinesterase as an indicator of liver metabolism and function showed steady improvement over the first 6-12 months for those patients surviving beyond 6 months. However, a decrease in cholinesterase near the end of the study was also noted. These fluctuating laboratory values lead to some speculation that the implanted cells may in some cases

not have been great enough in number, or may not have maintained viability or established meaningful functionality, sufficient to allow long-term supplementation of liver function. Alternatively, these fluctuations may be a natural long-term development of natural disease progression. It is expected that implants would not attain any level of functionality before 6 weeks post-implantation as the lack of vascularization limits the activity of the implanted cells. Prior to vascularization, the implants rely exclusively on diffused oxygen and nutrients. We can speculate that the implantation and vascularization processes may be expedited through the use of exogenous angiogenesis factors, and suggest that this be addressed in future studies.

Finally, according to the Karnofsky scale [17], most patients did experience a general improvement in condition (Figure 8). It is still not certain if this is related to the implanted cells or to natural improvement of the cirrhosis itself because the patients were still under conservative standard treatment for cirrhosis.

All patients in this study had limited potential for survival without liver transplant, and it is important to note that at no time were patients involved in this study prevented from receiving a liver transplant as a "rescue therapy by transplant". In fact, the procedure could be used as a bridge to transplant in selected patients.

However, at the time of the study there was no coordinated liver transplant program of any note in Indonesia, therefore the probability of a suitable donor being found was extremely low. In contrast, the German study either removed or maintained patients on the transplant list as there is a more effective transplant program in Germany [14].

Limitations

A number of limitations of this phase I study are noted. Importantly, as the study was primarily designed to assess safety, it was not sufficiently powered to determine significant clinical efficacy. Furthermore, placebo control arm in this trial would not have been ethically acceptable. Many physicians set a viral load limit of 10^4 iU/mL to define "inactive" hepatitis. We included patients with up to 3×10^5 and although we didn't detect any correlation between the viral load and patient outcome, we feel that future studies should apply a lower cut-off, as this is more likely to aid effective patient recovery. We acknowledge that cell viability and number of seeded cells with respect to the production of the tissue matrix are not discussed in this manuscript; they are the subject of a separate manuscript, as we focus on the clinical aspects of the technique in the current discussion. The single-center setting of this study might also be considered a limitation. This study was originally planned, and received all relevant ethical approvals, to be carried out in parallel in Indonesia and in Zurich, Switzerland. Unfortunately, due to logistical considerations we were unable to begin the study arm based in Switzerland.

Conclusion

We have shown for the first time in an ethically-approved clinical study in human that the transplantation of autologous hepatocyte tissue matrices for the treatment of end stage liver cirrhosis is a generally safe procedure, provided that patients are appropriately selected and monitored. Although the necessarily small size of our study limits our ability to show clear clinical efficacy, we do observe clinically relevant improvements in a number of clinical and laboratory indicators of liver function such as albumin and cholinesterase for some patients. Positive benefits were also seen in other measures for some patients with improvements in stamina, endurance to physical stress, frequency of hospitalization, incidence of encephalopathy, GI bleeding, ascites, and oesophageal varices.

Overall, we believe that the safety and clinical outcomes of this study represent sufficient data to pursue a phase II follow-up study that could prove the efficacy of this autologous cell transplantation technique more definitively. Stricter inclusion and exclusion criteria and a precisely defined and implemented follow-up protocols for the management of possible clinical risk factors and complications will greatly benefit the next phase of trials, as would more patients to provide greater statistical power.

Finally, recent advances in tissue matrix generation and coating technology have resulted in new matrix formulations that are expected to greatly improve autologous cell seeding and culturing and to enhance the clinical viability of implanted tissue.

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